

What is claimed is:

1. A method of performing RNA interference, said method comprising exposing an siRNA to a target nucleic acid, wherein said siRNA is comprised of a sense strand and an antisense strand, and wherein said sense strand is substantially nonfunctional.
2. The method according to claim 1, wherein said sense strand comprises at least one 2'-O-alkyl modification.
3. The method according to claim 2, wherein said sense strand comprises at least one cytosine- or uracil-containing nucleotide base, and said at least one cytosine- or uracil-containing nucleotide base has a 2'-O-methyl modification.
4. The method according to claim 2, wherein said 2'-O-alkyl modification is a 2'-O-methyl modification.
5. The method according to claim 4, wherein said at least one 2'-O-methyl modification is on the first, second, eighteenth and/or nineteenth nucleotide base.
6. The method according to claim 1, wherein the sense strand further comprises a 5' conjugate.
7. The method according to claim 6, wherein the conjugate is cholesterol.
8. The method according to claim 1, wherein the sense strand comprises a cap on its 3' end.
9. The method according to claim 8, wherein the cap is an inverted deoxythymidine or two consecutive 2'-O-methyl modified nucleotides.
10. The method according to claim 1, wherein said antisense strand comprises at least one modified nucleotide.
11. The method according to claim 10, wherein the at least one modified nucleotide is

a 2'-halogen-modified nucleotide.

12. The method according to claim 11, wherein the 2'-halogen modified nucleotide is a 2'-fluorine-modified nucleotide.

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13. The method according to claim 1, wherein the sense strand comprises one or more cytosine- and/or uracil-containing nucleotide bases, and each of said one or more cytosine- and/or uracil-containing nucleotide bases is 2'-fluorine modified.

10 14. A method of performing RNA interference, said method comprising exposing an siRNA to a target nucleic acid, wherein said siRNA comprises

(a) a conjugate;

(b) a sense strand comprising at least one 2'-O-alkyl modification, wherein said sense strand is substantially nonfunctional; and,

15 (c) an antisense strand comprising at least one 2'-fluorine modification, wherein said sense and antisense strands form a duplex of 18-30 base pairs.

15. The method according to claim 14, wherein said at least one 2'-O-alkyl modification is on the first, second, eighteenth and/or nineteenth nucleotide base.

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16. The method according to claim 14, wherein the conjugate is a 5' conjugate.

17. The method according to claim 14, wherein the conjugate is cholesterol.

25 18. The method according to claim 14, wherein the sense strand further comprises a cap on its 3' end.

19. The method according to claim 18, wherein the cap is an inverted deoxythymidine or two consecutive 2'-O-methyl modified nucleotides.

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20. A method of performing RNA interference, said method comprising exposing an siRNA to a target nucleic acid, wherein said siRNA is comprised of a sense strand and an antisense strand, and wherein at least one of said sense strand and said antisense strand comprises at least one orthoester modified nucleotide.

21. The method according to claim 20, wherein said at least one orthoester modified nucleotide is located on said sense strand.
- 5 22. The method according to claim 21, wherein the antisense strand comprises at least one nucleotide selected from the group consisting of a 2' halogen modified nucleotide, a 2' amine modified nucleotide, a 2'-O-alkyl modified nucleotide and a 2' alkyl modified nucleotide.
- 10 23. The method according to claim 22, wherein the antisense strand comprises at least one 2' halogen modified nucleotide and said halogen is fluorine.
24. The method according to claim 21, wherein the siRNA further comprises a conjugate.
- 15 25. The method according to claim 24, wherein said conjugate is selected from the group consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof.
- 20 26. The method according to claim 24, wherein the conjugate is cholesterol.
27. The method according to claim 24, wherein conjugate is polyethylene glycol.
- 25 28. The method according to claim 20, wherein the siRNA comprises 18-30 nucleotide base pairs.
29. The method according to claim 28, wherein the siRNA comprises 19 nucleotide base pairs.
- 30 30. The method according to claim 20, wherein the siRNA has an overhang of at least one nucleotide unit on at least one of said sense strand and said antisense strand.
31. The method according to claim 20, wherein at least one strand of the siRNA

comprises at least one modified internucleotide linkage.

32. The method according to claim 31, wherein the modified internucleotide linkage is selected from the group consisting of a phosphorothioate linkage and a
5 phosphorodithioate linkage.

33. The method according to claim 20, wherein at least one strand of the siRNA is a polyribonucleotide.

10 34. A method of performing RNA interference, said method comprising exposing an siRNA to a target nucleic acid, wherein said siRNA is comprised of:

- (a) a sense strand,
- (b) an antisense strand, and
- (c) a conjugate,

15 wherein at least one of said sense strand and said antisense strand comprises a 2' modified nucleotide.

35. An siRNA, comprising:

- 20 (a) a sense strand, wherein said sense strand comprises a polynucleotide that is comprised of at least one orthoester modified nucleotide; and
- (b) an antisense strand, wherein said antisense strand comprises a polynucleotide that is comprised of at least one 2' modified nucleotide.

36. The siRNA of claim 35, wherein the antisense strand comprises at least one
25 nucleotide selected from the group consisting of a 2' halogen modified nucleotide, a 2' amine modified nucleotide, a 2'-O-alkyl modified nucleotide and a 2' alkyl modified nucleotide.

37. The siRNA of claim 36, wherein the 2' modified nucleotide is a 2' halogen
30 modified nucleotide and said halogen is fluorine.

38. The siRNA of claim 35, further comprising a conjugate.

39. The siRNA of claim 38, wherein said conjugate is selected from the group

consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof.

40. The siRNA of claim 38, wherein said conjugate is cholesterol.

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41. The siRNA of claim 38, wherein said conjugate is polyethylene glycol.

42. The siRNA of claim 35, wherein said siRNA is comprised of 18-30 nucleotide base pairs.

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43. The siRNA of claim 42, wherein said siRNA is comprised of 19 nucleotide base pairs.

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44. The siRNA of claim 35, further comprising an overhang of at least one nucleotide unit on at least one of said sense strand and said antisense strand.

45. The siRNA of claim 35, wherein at least one of said sense strand and said antisense strand comprises at least one modified internucleotide linkage.

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46. The siRNA of claim 45, wherein the modified internucleotide linkage is selected from the group consisting of a phosphorothioate linkage and a phosphorodithioate linkage.

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47. The siRNA of claim 35, wherein at least one of said sense strand and said antisense strand is a polyribonucleotide.

48. An siRNA, comprising:

(a) a sense strand, wherein said sense strand comprises a polynucleotide that is comprised of at least one orthoester modified nucleotide;

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(b) an antisense strand, wherein said antisense strand comprises a polynucleotide that is comprised of at least one 2' modified nucleotide; and

(c) a conjugate.

49. The siRNA of claim 48, wherein the conjugate is located on the sense strand.

50. The siRNA of claim 48, wherein the conjugate is located on the antisense strand.
51. The siRNA of claim 48, wherein the antisense strand comprises at least one
5 nucleotide selected from the group consisting of a 2' halogen modified nucleotide, a 2'
amine modified nucleotide, a 2'-O-alkyl modified nucleotide and a 2' alkyl modified
nucleotide.
52. The siRNA of claim 51, wherein the sense strand is comprised of a 2' halogen
10 modified nucleotide and said halogen is fluorine.
53. The siRNA of claim 48, wherein the conjugate is selected from the group
consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates,
lipids, polymers, nucleotides, polynucleotides, and combinations thereof.
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54. The siRNA of claim 48, wherein the conjugate is cholesterol.
55. The siRNA of claim 48, wherein the conjugate is polyethylene glycol.
- 20 56. The siRNA of claim 48, wherein said siRNA is comprised of 18-30 nucleotide base
pairs.
57. The siRNA of claim 56, wherein said siRNA is comprised of 19 nucleotide base
pairs.
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58. The siRNA of claim 48, further comprising an overhang of at least one nucleotide
unit on at least one of said sense strand and said antisense strand.
59. The siRNA of claim 48, wherein at least one of said sense strand and said antisense
30 strand comprises at least one modified internucleotide linkage.
60. The siRNA of claim 59, wherein the modified internucleotide linkage is selected
from the group consisting of a phosphorothioate linkage and a phosphorodithioate
linkage.

61. The siRNA of claim 48, wherein at least one of said sense strand and said antisense strand is a polyribonucleotide.
- 5 62. An siRNA, comprising:
- (a) a sense strand comprised of at least one orthoester modified nucleotide;
 - (b) an antisense strand; and
 - (c) a conjugate.
- 10 63. The siRNA of claim 62, wherein said conjugate is located on the sense strand.
64. The siRNA of claim 62, wherein said is located on the antisense strand.
65. The siRNA of claim 62 wherein the antisense strand comprises at least one
- 15 nucleotide selected from the group consisting of a 2' halogen modified nucleotide, a 2' amine modified nucleotide, a 2'-O-alkyl modified nucleotide and a 2' alkyl modified nucleotide.
66. The siRNA of claim 65, wherein the antisense strand is comprised of a 2' halogen
- 20 modified nucleotide and said halogen is fluorine.
67. The siRNA of claim 62, wherein the conjugate is selected from the group consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof.
- 25 68. The siRNA of claim 62, wherein the conjugate is cholesterol.
69. The siRNA of claim 62, wherein the conjugate is polyethylene glycol.
- 30 70. The siRNA of claim 62, wherein the siRNA is comprised of 18-30 nucleotide base pairs.
71. The siRNA of claim 70, wherein the siRNA is comprised of 19 nucleotide base pairs.

72. The siRNA of claim 62, further comprising an overhang of at least one nucleotide unit on at least one of said sense strand and said antisense strand.

5 73. The siRNA of claim 62, wherein at least one of said sense strand and said antisense strand comprises at least one modified internucleotide linkage.

74. The siRNA of claim 62, wherein at least one of said sense strand and said antisense strand is a polyribonucleotide.

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75. An siRNA, comprising:

- (a) a sense strand;
- (b) an antisense strand; and
- (c) a conjugate;

15 wherein the sense strand and/or the antisense strand comprises at least one 2' modified nucleotide.

76. The siRNA of claim 75, wherein the 2' modified nucleotide is selected from the group consisting of a 2' halogen modified nucleotide, a 2' amine modified nucleotide, a
20 2'-O-alkyl modified nucleotide and a 2' alkyl modified nucleotide.

77. The siRNA of claim 76, wherein the 2' modified nucleotide is a 2' halogen modified nucleotide and said halogen is fluorine.

25 78. The siRNA of claim 75, wherein the conjugate is selected from the group consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof.

79. The siRNA of claim 75, wherein the conjugate is cholesterol.

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80. The siRNA of claim 75, wherein the conjugate is polyethylene glycol.

81. The siRNA of claim 75, wherein said siRNA is comprised of 18-30 nucleotide base pairs.

82. The siRNA of claim 75, wherein said siRNA is comprised of 19 nucleotide base pairs.

5 83. The siRNA of claim 75, further comprising an overhang of at least one nucleotide unit on at least one of said sense strand and said antisense strand.

84. The siRNA of claim 75, wherein at least one of said sense strand and said antisense strand comprises at least one modified internucleotide linkage.

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85. The siRNA of claim 84, wherein the modified internucleotide linkage is selected from the group consisting of a phosphorothioate linkage and a phosphorodithioate linkage.

15 86. The siRNA of claim 75, wherein at least one of said sense strand and said antisense strand is a polyribonucleotide.

87. An siRNA, comprising:

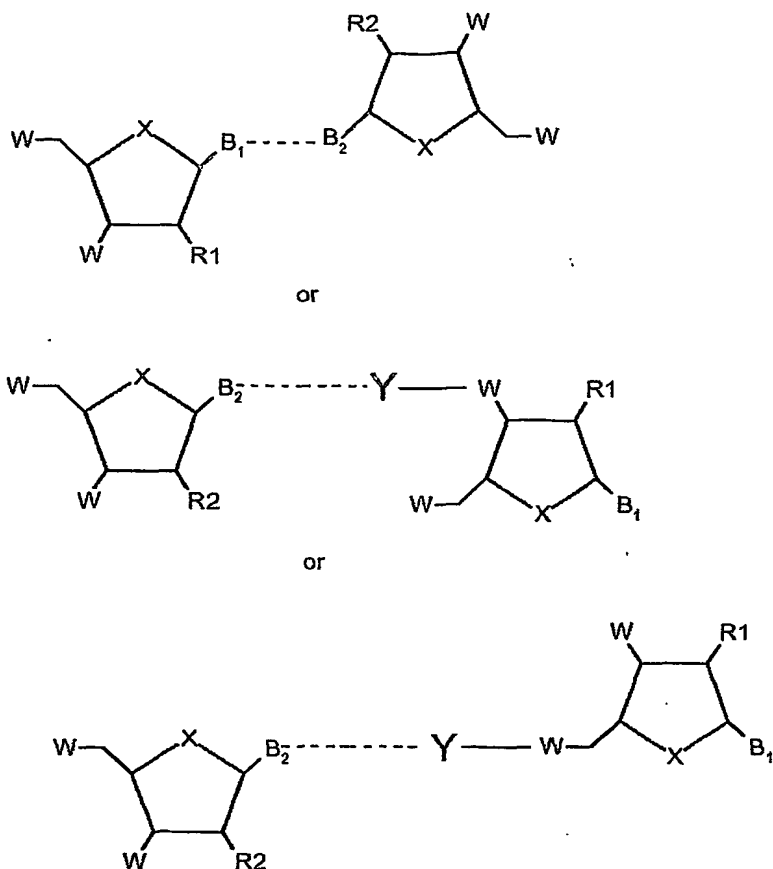
20 (a) a sense strand, wherein said sense strand is comprised of at least one 2' orthoester modified nucleotide;

(b) an antisense strand, wherein said antisense strand is comprised of at least one 2' modified nucleotide selected from the group consisting of a 2' halogen modified nucleotide, a 2' amine modified nucleotide, a 2'-O-alkyl modified nucleotide, and a 2' alkyl modified nucleotide; and

25 (c) a conjugate selected from the group consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof;
wherein said polyribonucleotide comprises between 18 and 30 nucleotide base pairs.

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88. A composition comprising:



wherein:

5 each of B₁ and B₂ is a nitrogenous base, heterocycle or carbocycle;

X is selected from the group consisting of O, S, C, and N;

W is selected from the group consisting of an OH, a phosphate, a phosphate ester, a phosphodiester, a phosphotriester, a modified internucleotide link, a conjugate, a nucleotide, and a polynucleotide;

10 R1 is an orthoester;

R2 is selected from the group consisting of a 2'-O-alkyl group, an alkyl group, and amine, and a halogen; and

Y is a nucleotide or polynucleotide.

15 89. An siRNA comprised of:

(a) a sense strand, wherein said sense strand is comprised of

- 5
- (i) a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2'-O-alkyl sense modification and said second 5' terminal sense nucleotide comprises a second 2'-O-alkyl sense modification;
- (ii) at least one 2'-O-alkyl pyrimidine modified sense nucleotide, wherein said at least one 2'-O-alkyl pyrimidine modified sense nucleotide is a nucleotide other than said first 5' terminal sense nucleotide or said second 5' terminal sense nucleotide; and
- 10 (b) an antisense strand, wherein said antisense strand is comprised of
- (i) at least one 2' halogen modified pyrimidine nucleotide; and
- (ii) a first 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide is phosphorylated at its 5' carbon position,
- 15 wherein the sense strand and the antisense strand are capable of forming a duplex of between 18 and 30 base pairs.

20 90. The siRNA of claim 89, wherein the sense strand and antisense strand are capable of forming a duplex of between 19 and 25 base pairs.

91. The siRNA of claim 89 further comprising a label.

25 92. The siRNA of claim 91, wherein said label is attached to said first 5' terminal sense nucleotide.

93. The siRNA of claim 92, wherein said label is a fluorescent dye.

30 94. The siRNA of claim 89, wherein all pyrimidines on said sense strand comprise a 2'-O-alkyl modification.

95. The siRNA of claim 89, wherein all pyrimidines on said antisense strand comprise a 2'-fluoro modification.

96. The siRNA of claim 89, wherein said first 2'-O-alkyl sense modification, said second 2'-O-alkyl sense modification, and said at least one 2'-O-alkyl pyrimidine modified sense nucleotide each comprises 2'-O-methyl.

5 97. The siRNA of claim 89, wherein said halogen is fluorine.

98. The siRNA of claim 89, wherein the first 2'-O-alkyl sense modification is 2'-O-methyl, the second 2'-O-alkyl sense modification is 2'-O-methyl, the at least one 2'-O-alkyl pyrimidine modified sense nucleotide comprises 2'-O-methyl, the halogen is
10 fluorine, and the first 5' terminal sense nucleotide further comprises a fluorescent dye.

99. The siRNA of claim 89 further comprising at least one phosphorothioate.

100. The siRNA of claim 89 further comprising at least one methylphosphonate.

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101. An siRNA comprised of:

(a) a sense strand, wherein said sense strand is comprised of

(i) a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide
20 comprises a first 2'-O-alkyl sense modification and said second 5' terminal sense nucleotide comprises a second 2'-O-alkyl sense modification;

(ii) at least one 2'-O-alkyl pyrimidine modified sense nucleotide, wherein said at least one 2'-O-alkyl pyrimidine modified sense
25 nucleotide is a nucleotide other than said first 5' terminal sense nucleotide or said second 5' terminal sense nucleotide; and

(b) an antisense strand, wherein said antisense strand is comprised of

(i) a first 5' terminal antisense nucleotide and a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense
30 nucleotide comprises and a first 2'-O-alkyl antisense modification and said second 5' terminal antisense nucleotide comprises a second 2'-O-alkyl antisense modification; and
(ii) at least one 2'-O-alkyl pyrimidine modified antisense nucleotide, wherein said at least one 2'-O-alkyl modified antisense

nucleotide is a nucleotide other than said first 5' terminal antisense nucleotide or said second 5' terminal antisense nucleotide,

5 wherein the sense strand and antisense strand are capable of forming a duplex of between 16 and 28 base pairs.

102. The siRNA of claim 101, wherein the sense strand and antisense strand are capable of forming a duplex of 19-28 base pairs.

10 103. The siRNA of claim 101, wherein the sense strand and antisense strand are capable of forming a duplex of 19-25 base pairs.

104. The siRNA of claim 101, wherein all pyrimidines on said sense strand comprise a 2'-O-alkyl modification.

15 105. The siRNA of claim 101, wherein all pyrimidines on said antisense strand comprise a 2'-O-alkyl modification.

20 106. The siRNA of claim 101, wherein said first 2'-O-alkyl sense modification, said second 2'-O-alkyl sense modification, said first 2'-O-alkyl antisense modification, said second 2'-O-alkyl antisense modification, said at least one 2'-O-alkyl pyrimidine modified sense nucleotide, and said at least one 2'-O-alkyl pyrimidine modified antisense nucleotide each comprises 2'-O-methyl.

25 107. The siRNA of claim 101 further comprises a label.

108. The siRNA of claim 107, wherein said label is attached to said first 5' terminal sense nucleotide.

30 109. The siRNA of claim 107, wherein said label is a fluorescent dye.

110. The siRNA of claim 101 further comprising at least one phosphorothioate.

111. The siRNA of claim 101 further comprising at least one methylphosphonate.

112. A method of gene silencing comprising introducing the siRNA of claim 89 into a cell that is expressing or is capable of expressing a target gene.

5 113. A method of performing gene silencing comprising using a control reagent, wherein said control reagent is the siRNA of claim 101, and introducing said control reagent into a cell that is expressing or is capable of expressing a target gene.

114. An siRNA, comprising:

10 (a) an antisense strand, wherein said antisense strand is comprised of a first 5' terminal antisense nucleotide and said first 5' terminal antisense nucleotide is phosphorylated at said first 5' terminal antisense nucleotide's 5' carbon position; and

(b) a sense strand, wherein said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5'
15 terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification.

115. The siRNA of claim 114, wherein each of said first 2' carbon sense modification and said second 2' carbon sense modification is a 2'-O-alkyl modification.
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116. The siRNA of claim 115, further comprising a third 5' terminal sense nucleotide, wherein said third 5' terminal sense nucleotide comprises a 2'-O-alkyl modification.

117. The siRNA of claim 115, wherein at least one of the 2'-O-alkyl modifications is a
25 2'-O-methyl modification.

118. The siRNA of claim 115, wherein each of the 2'-O-alkyl modifications is a 2'-O-methyl modification.

30 119. The siRNA of claim 115, wherein said siRNA is comprised of between 18 and 30 base pairs.

120. The siRNA of claim 119, wherein said siRNA is comprised of between 19 and 25 base pairs.

121. The siRNA of claim 119, wherein said antisense strand and said sense strand are substantially complementary and form a duplex that does not contain overhangs.

5 122. A method of reducing off-target effects during RNAi, said method comprising exposing an siRNA to a target nucleic acid, wherein said siRNA comprises an antisense strand and a sense strand, wherein:

(a) said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, and wherein said first 5' terminal sense nucleotide
10 comprises a first 2' carbon modification and said second 5' terminal sense nucleotide comprises a second 2' carbon modification; and

(b) said antisense strand is comprised of a first 5' terminal antisense nucleotide and said first 5' terminal antisense nucleotide is phosphorylated at said first 5' terminal antisense nucleotide's 5' carbon position.

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123. The method according to claim 122, wherein each of said first 2' carbon sense modification and said second 2' carbon sense modification is a 2'-O-alkyl modification.

124. The method according to claim 123, further comprising a third 5' terminal sense
20 nucleotide, wherein said third 5' terminal sense nucleotide comprises a 2'-O-alkyl modification.

125. The method according to claim 123, wherein at least one of the 2'-O-alkyl modifications is a 2'-O-methyl modification.

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126. The method according to claim 123, wherein each of the 2'-O-alkyl modifications is a 2'-O-methyl modification.

127. The method according to claim 122, wherein said siRNA is comprised of between
30 18 and 30 base pairs.

128. The siRNA of claim 127, wherein said siRNA is comprised of between 19 and 25 base pairs.

129. The method according to claim 127, wherein said antisense strand and said sense strand are substantially complementary and form a duplex that does not contain overhangs.

5 130. A unimolecular siRNA capable of forming a hairpin, said unimolecular siRNA comprising:

(a) an antisense region, wherein said antisense region is comprised of a first 5' terminal antisense nucleotide and said first 5' terminal antisense nucleotide is phosphorylated at said first 5' terminal antisense nucleotide's 5' carbon position;

10 (b) a sense region, wherein said sense region is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification; and

15 (c) a loop region, wherein said loop region is located between said sense region and said antisense region.

131. The siRNA of claim 130, wherein each of said first 2' carbon sense modification and said second 2' carbon sense modification is a 2'-O-alkyl modification.

20 132. The siRNA of claim 130, further comprising a third 5' terminal sense nucleotide, wherein said third 5' terminal sense nucleotide comprises a 2'-O-alkyl modification.

133. The siRNA of claim 131, wherein at least one of the 2'-O-alkyl modifications is a 2'-O-methyl modification.

25 2'-O-methyl modification.

134. The siRNA of claim 131, wherein each of the 2'-O-alkyl modifications is a 2'-O-methyl modification.

30 135. The siRNA of claim 130, wherein said siRNA is comprised of between 18 and 30 base pairs.

136. The siRNA of claim 135, wherein said siRNA is comprised of between 19 and 25 base pairs.

137. A method for reducing off-target effects during RNAi, said method comprising:
exposing a unimolecular siRNA that is capable of forming a hairpin to a target nucleic
acid, wherein said unimolecular siRNA comprises an antisense region and a sense
5 region, wherein:

(a) said sense region is comprised of a first 5' terminal sense nucleotide and a
second 5' terminal sense nucleotide, and wherein said first 5' terminal sense nucleotide
comprises a first 2' carbon sense modification and said second 5' terminal sense
nucleotide comprises a second 2' carbon sense modification;

10 (b) said antisense region is comprised of a first 5' terminal antisense nucleotide
and said first 5' terminal antisense nucleotide is phosphorylated at said first 5'
terminal antisense nucleotide's 5' carbon position; and

(c) a loop region, wherein said loop region is located between said sense region
and said antisense region.

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138. The method according to claim 137, wherein each of said first 2' carbon sense
modification and said second 2' carbon sense modification is a 2'-O-alkyl modification.

139. The method according to claim 138, further comprising a third 5' terminal sense
20 nucleotide, wherein said third 5' terminal sense nucleotide comprises a 2'-O-alkyl
modification.

140. The method according to claim 138, wherein at least one of the 2'-O-alkyl
modifications is a 2'-O-methyl modification.

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141. The method according to claim 138, wherein each of the 2'-O-alkyl modifications
is a 2'-O-methyl modification.

142. The method according to claim 137, wherein said siRNA is comprised of between
30 18 and 30 base pairs.

143. The siRNA of claim 114 further comprising a second 5' terminal antisense
nucleotide, wherein said first 5' terminal antisense nucleotide further comprises a first

2' carbon terminal antisense modification and said second 5' terminal antisense nucleotide comprises a second 2' carbon terminal antisense modification.

5 144. The unimolecular siRNA of claim 130 further comprising a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide further comprises a first 2' carbon terminal antisense modification and said second 5' terminal antisense nucleotide comprises a second 2' carbon terminal antisense modification.

10 145. A method of reducing off-target effects induced during RNAi, comprising introducing the siRNA of any one of claims 114 or 143 into a cell.

146. A method of reducing off-target effects during RNAi comprising introducing the siRNA of claim 144 to a cell.

15 147. An siRNA, comprising:

(a) an antisense strand, wherein said antisense strand is comprised of a first 5' terminal antisense nucleotide and a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide comprises a first 2' carbon antisense modification and said second 5' terminal antisense nucleotide comprises a second 2' carbon antisense modification; and

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(b) a sense strand, wherein said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification.

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148. The siRNA of claim 147, wherein the sense strand and the antisense strand form a duplex of from 16–28 base pairs.

30 149. The siRNA of claim 148, wherein the sense strand and the antisense strand form a duplex of from 18–25 base pairs.

150. The siRNA of claim 148, wherein said first 2' carbon antisense modification, said second 2' carbon antisense modification, said first 2' carbon sense modification and said second 2' carbon sense modification each comprises a 2'-O-alkyl modification.
- 5 151. The siRNA of claim 150, wherein the 2'-O-alkyl modification is 2'-O-methyl.
152. The siRNA of claim 150, further comprising a label.
153. A unimolecular siRNA capable of forming a hairpin, said unimolecular siRNA
10 comprising:
- (a) an antisense region, wherein said antisense region is comprised of a first 5' terminal antisense nucleotide and a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide comprises a first 2' carbon antisense modification and said second 5' terminal antisense nucleotide comprises a second 2'
15 carbon antisense modification;
- (b) a sense region, wherein said sense region is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification;
20 and
- (c) a loop region, wherein said loop region is located between said sense region and said antisense region.
154. The siRNA of claim 153, wherein the sense region and the antisense region form
25 a duplex of from 16–28 base pairs.
155. The siRNA of claim 154, wherein the sense region and the antisense region form a duplex of from 18–25 base pairs.
- 30 156. The siRNA of claim 154, wherein said first 2' carbon antisense modification, said second 2' carbon antisense modification, said first 2' carbon sense modification and said second 2' carbon sense modification each comprises a 2'-O-alkyl modification.
157. The siRNA of claim 156, wherein the 2'-O-alkyl modification is 2'-O-methyl.

158. The siRNA of claim 156, further comprising a label.
159. An siRNA, comprising a sense strand and an antisense strand, wherein the sense
5 strand and the antisense strand each comprises at least one orthoester modification at a 2' position.
160. An siRNA, comprising a sense strand and an antisense strand, wherein said
10 antisense strand comprises at least one orthoester modification and/or at least one modification selected from the group consisting of a 2'-halogen modification, a 2'-alkyl modification, a 2'-O-alkyl modification, a 2'-amine modification, and a 2'-deoxy modification.
161. An siRNA, comprising a sense strand and an antisense strand, wherein the sense
15 strand and/or the antisense strand comprises at least one orthoester modification, and wherein the sense strand and/or the antisense strand comprises at least one 2' modification selected from the group consisting of a 2'-halogen modification, a 2'-alkyl modification, a 2'-O-alkyl modification, a 2'-amine modification, and a 2'-deoxy modification.
- 20 162. An siRNA, comprising:
- (a) a sense strand, wherein said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said
25 second 5' terminal sense nucleotide comprises a second 2' carbon sense modification; and
- (b) an antisense strand, wherein said antisense strand is comprised of a first 5' terminal antisense nucleotide and a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide comprises a first 2' carbon antisense
30 modification and a 5' modification, and said second 5' terminal antisense nucleotide comprises a second 2' carbon antisense modification.
163. An siRNA, comprising a sense strand and an antisense strand, wherein the antisense strand comprises at least one 2'-orthoester modification, and wherein the

antisense strand further modified with one or more modifications selected from the group consisting of a 2' orthoester modification, a 2'-alkyl modification, a 2'-halogen modification, a 2'-O-alkyl modification, a 2'-amine modification, and a 2'-deoxy modification, wherein the 2'-alkyl modification, the 2'-O-methyl modification, and the
5 2' halogen modification is on one or more pyrimidines of the antisense strand, and wherein the siRNA further comprises a 3' cap.

164. The siRNA of claim 162, wherein the 2' carbon modification is a 2'-O-alkyl modification.
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165. The siRNA of claim 164, wherein the 2'-O-alkyl modification is a 2'-O-methyl modification.

166. The siRNA of claim 162, wherein the first 5' terminal sense nucleotide and/or the
15 first 5' terminal antisense nucleotide is further modified with a 5' blocking group.

167. The siRNA of claim 166, wherein the 5' blocking group is selected from the group consisting of a 5'-methyl modification, a 5'-O-methyl modification, and a 5' azide modification.
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168. The siRNA according to any of claims 101-111, wherein the first and second 2'-O-alkyl modifications of the 5' terminal sense nucleotide and/or the 5' terminal antisense nucleotide is a 2'-O-methyl modification, and the first 5' terminal sense and/or antisense nucleotide further comprises a 5' blocking group.
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169. The siRNA of claim 168, wherein the 5' blocking group is selected from the group consisting of a 5'-methyl modification, a 5'-O-methyl modification, or a 5' azide modification.

30 170. The siRNA of any of claims 35-111, 114-121, 130-136, 143-144, 147-162, further comprising a 3' cap.

171. The siRNA of claim 170, wherein the 3' cap is an inverted deoxythymidine.

172. The siRNA of any of claims 35-111, 114-121, 130-136, 143-144, and 147-161, further comprising at least one of a 2'-deoxy modification and/or a methylphosphonate internucleotide linkage.
- 5 173. The siRNA of any of claims 35-111, 114-121, 130-136, 143-144, and 147-161, further comprising on one or more pyrimidines a modification selected from the group consisting of a 2'-alkyl modification, a 2'-O-alkyl modification, and a 2'-halogen modification.
- 10 174. The siRNA of claim 175, wherein the 2'-alkyl modification is a 2'-methyl modification.
175. The siRNA of claim 175, wherein the 2'-O-alkyl modification is a 2'-O-methyl modification.
- 15 176. The siRNA of claim 175, wherein the 2'-halogen modification is a 2'-fluorine modification.
177. The siRNA of any of claims 87-111, 114-121, 130-136, 143-144, 147-178, further comprising a conjugate.
- 20 178. The siRNA of claim 179, wherein the conjugate is selected from the group consisting of amino acids, peptides, polypeptides, proteins, sugars, carbohydrates, lipids, polymers, nucleotides, polynucleotides, and combinations thereof.
- 25 179. The siRNA of claim 179, wherein the conjugate comprises cholesterol or PEG.
180. The siRNA of claim 179, wherein the conjugate further comprises a label.
- 30 181. The siRNA of claim 182, wherein the label is a fluorescent label.
182. The siRNA of claim 183, wherein the fluorescent label is selected from the group consisting of TAMRA, BODIPY, Cy3, Cy5, fluorescein, and Dabsyl.

183. The siRNA of any of claims 38-41 and 48-86, wherein the conjugate further comprises a label.

184. The siRNA of claim 185, wherein the label is a fluorescent label.

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185. The siRNA of claim 186, wherein the fluorescent label is selected from the group consisting of TAMRA, BODIPY, Cy3, Cy5, fluorescein, and Dabsyl

186. The siRNA of any of claims 36, 51, 65, 76, 87, and 88, wherein the 2'-alkyl modified nucleotide is a 2'-methyl modified nucleotide.

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187. The siRNA of any of claims 36, 51, 65, 76, 87, and 88, wherein the 2'-O-alkyl modified nucleotide is a 2'-O-methyl modified nucleotide.

188. The siRNA of any of claims 36, 51, 65, 76, 87, and 88, wherein the 2'-amine modified nucleotide is a 2'-NH₂ modified nucleotide.

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189. The siRNA of any of claims 87 and 88, wherein the 2'-halogen modified nucleotide is a 2'-fluorine modified nucleotide.

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190. The siRNA of any of claims 45, 59, 73, 84 and 88, wherein the modified internucleotide link is a methylphosphonate.

191. The siRNA of claim 89, further comprising at least one modified internucleotide link.

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192. The siRNA of claim 193, wherein the at least one modified internucleotide link is selected from the group consisting of a phosphorothioate link, a phosphorodithioate link, a methylphosphonate link, and combinations thereof.

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193. The siRNA of any of claims 88-111, 114-121, 130-136, 147-158, and 162, further comprising at least one 2'-orthoester modification.

194. The siRNA of claim 195, wherein the 2'-orthoester modification is a 2'-

bis(hydroxy ethyl) orthoester modification.

195. The siRNA of any of claims 35-74, 87-88, 159-161, and 163, wherein the 2' orthoester modification is a 2'-bis(hydroxy ethyl) orthoester.

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196. A method of performing RNA interference, comprising administering the siRNA of any of claims 35-74, 87-88, 159-161, and 163-194 to a cell.

197. The method of claim 198, wherein the orthoester modification is a 2'-bis(hydroxy ethyl) orthoester.

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198. A method of performing RNA interference, comprising administering the siRNA of any of claims 195-199 to a cell.

15 199. An siRNA, comprising:

(a) an antisense strand; and

(b) a sense strand, wherein said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification.

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200. An siRNA, comprising:

(a) a sense strand, wherein said sense strand is comprised of a first 5' terminal sense nucleotide and a second 5' terminal sense nucleotide, wherein said first 5' terminal sense nucleotide comprises a first 2' carbon sense modification and said second 5' terminal sense nucleotide comprises a second 2' carbon sense modification; and

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(b) an antisense strand, wherein said antisense strand is comprised of a first 5' terminal antisense nucleotide and a second 5' terminal antisense nucleotide, wherein said first 5' terminal antisense nucleotide comprises a 5' phosphate modification and said second 5' terminal antisense nucleotide comprises a 2' carbon antisense modification.

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